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USSR WORK ON APPLICATION OF ULTRASOUND WAVES IN BIOLOGY

I. Ye. El'piner

A property of ultrasound waves important for biological applications is that these waves can rupture cells and cell structures. From the effect of ultrasound waves, red blood corpuscles, erythrocytes, leucocytes, epithelial cells, liver cells, and other cells suspended in a physiological salt solution are destroyed. As a result of the action of ultrasound waves, almost all microorganisms perish, including pathogenic and nonpathogenic microorganisms, aerobic and anaerobic bacteria, bacteria which are located individually as well as in pairs or in chains, cocci, etc.

A more detailed check on the effects can be obtained by observation under an electronmicroscope. It has been shown that yeast cells are destroyed by the action of ultrasound waves. Tuberculosis bacilli, which are resistant to many physical agents, are also subjected to mechanical rupture in an ultrasound field.

Ultrasound waves also destroy viruses. The manner in which the outer covering of cells is ruptured varies, depending on the structure of this covering. In some cases, the covering is torn into individual small particles; in other cases, defects are produced in the covering, mainly holes through which the contents of the cells penetrate into the surrounding medium.

The question arises as to whether all microorganisms and animal and plant cells perish immediately after the liquid in which they are present is exposed to ultrasound waves. Experiments show that a considerable length of time is necessary for complete sterilization of the liquid.

Of importance in this connection are the biological characteristics of the objects treated, which determine their sensitivity to ultrasound.

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The action of ultrasound also depends on the number of cells being irradiated. When the suspension of bacteria is highly concentrated, the action of ultrasound waves is less effective. The same relationship is found in connection with the hemolizing effect of ultrasound; disintegration of red blood corpuscles at high concentrations (one million corpuscles per cubic millimeter) takes place very slowly. On the other hand, at lower concentrations (20,000 - 160,000 red blood corpuscles per cubic millimeter) almost all erythrocytes are destroyed within a very short time. Naturally, this effect also depends, to a considerable extent, on the intensity of irradiation.

In addition, it should be emphasized that when an individual cell is ruptured in an ultrasound field, this rupture or destruction almost always takes place instantaneously, in other words, in less than 1/1,000th of a second. Under such conditions, the contents of the cell pass into the surrounding medium before any destructive effect on the medium has been produced by the ultrasound waves. In this manner, biologically active substances which are contained in the cells -- vitamins, enzymes, toxins, etc., -- are extracted. Thus, it has been possible to extract from *Proteus vulgaris* a number of enzymes which bring about oxidation of naturally occurring amino acids, such as leucine, tryptophan, tyrosine, and others.

From a culture of *Cl. histolyticum*, the enzyme desaminase was extracted. This enzyme acts on aspartic acid, transforming it into succinic acid. Using ultrasound waves, it was possible to extract from purpureal bacteria the pigment called bacteriochlorophyll, as well as compounds of the carotenoid class.(1) In collaboration with A. P. Sheynker, we succeeded in extracting the endotoxin from whooping cough bacilli.(2) This endotoxin, on standing, lost its toxic properties but retained its immunogenic properties.

It is necessary to note that the extraction of the substances mentioned by means of an ultrasound field, takes place without the necessity of the addition of chemicals, at a relatively low temperature, and under strictly sterile conditions. However, further investigations showed that on longer exposure to irradiation with ultrasound waves, biologically active substances are usually subjected to destruction after they have been extracted from the cells as a result of the action of ultrasound waves. More detailed investigation of this phenomenon brought out some interesting facts. It was established that as a result of the action of ultrasound waves, processes take place which under ordinary conditions are produced by specific catalysts and enzymes or which occur as the result of the addition of strongly acting chemical agents. Thus, in the ultrasound field, disruption of protein particles is observed. As a result of the action of ultrasound waves, amino acids, which enter into the composition of protein molecules, are also destroyed. At the same time, formation of aspartic acid as a result of the destruction of histidine was observed.

As a result of the action of ultrasound waves, nucleic acids are split. Furthermore, the components of nucleic acid, namely purine and pyrimidine bases, are also subjected to fission. As a result of the action of ultrasound waves, there is also fission of other complex molecules. Among these are hyaluronic and chondroitin sulphuric acids, which commonly occur in connective tissues and in liquid constituents of the animal organism in general. These substances are of considerable importance in physiological processes. We may conclude that these acids are decomposed as a result of the reduction of the relative viscosity of their aqueous solutions after the solutions have been subjected to the action of ultrasound waves.(3) It is widely assumed that bile pigments (bilirubin and biliverdin) are formed in the organism as the result of the decomposition of protoporphyrins. However, it has been impossible up to now to reproduce a decomposition of protoporphyrin in the laboratory. The process could be carried out, however, by exposing an aqueous solution of protoporphyrin IX to ultrasound waves. In the solution that had been irradiated, bilirubin, biliverdin, and individual pyrroles were found.(4)

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Different substances behave differently, depending on their structure, as far as their decomposition in the ultrasound field is concerned. Some dye-stuffs proved to be sensitive to ultrasound waves. For instance, methylene blue is subjected to rapid decomposition through the action of ultrasound waves.

The mechanical forces which develop in connection with the formation and bursting of cavitation bubbles have received considerable attention in work on the biological effects of ultrasound waves. However, the electrochemical processes, which also take place in aqueous solution exposed to ultrasound waves, must likewise be subjected to study. These electrochemical phenomena are probably connected with the fact that high electric potentials arise in the region of cavitation. The fact that high potentials arise in connection with cavitation has been pointed out by V. L. Levshina and S. N. Rzhevskina who have studied the luminescence of aqueous solutions exposed to ultrasound waves.(5) The same conclusion was reached by Ya. I. Frenkel', who has indicated as highly probable the fact that transition of electrons takes place in cavitation gas bubbles during the initial stage of their formation.(6) This electron transition is accompanied by ultraviolet radiation.

In Frenkel's opinion, this is the explanation of the luminescence of aqueous solutions during the course of typical reactions which take place in the ultrasound field. On the other hand, there are reasons to believe that freely moving electric charges ionize water molecules which are present in cavitation spaces in the form of water vapor. The ionized water molecules are split into free hydroxyl radicals and atomic hydrogen which possess a high degree of chemical reactivity. From this standpoint, a number of phenomena in ultrasound fields becomes understandable. Thus, it becomes clear why chemical processes produced by ultrasound waves take place predominantly in an aqueous medium. Furthermore, the so-called protective action of extraneous substances which are present in a solution becomes understandable. For instance, it has been established that tryptophan suppresses the oxidation of iodine in an ultrasound field.(7) When methionine or leucine is present in a solution, the decomposition of purine and pyrimidine bases is inhibited. A mixture of methyl and ethylalcohols protects an aqueous solution of methylene blue from discoloration.(8) A protective action is exerted by substances which themselves decompose as a result of the action of ultrasound. In other words, a protective action is exerted by substances which interact with the chemically active products of the fission of water. For that reason, in the presence of protective substances, those chemical reactions are inhibited the occurrence of which is ordinarily caused by the participation of the chemically active substances in question (i.e., of the products of ionization of water). The phenomenon of protective action opens up new possibilities for controlled application of this phenomenon.

Of exceptional interest is the problem of the exact mechanism of the action of ultrasound waves on the complete organism. Ultrasound waves penetrate deeply into the body. When their intensity is great, they produce considerable destruction in all organs and tissues. Thus, a bundle of waves directed toward the abdominal region of the rabbit brings about, along its path, rupture of blood vessels of the skin, point hemorrhages in the peritoneal regions affected, and multiple perforations of the intestine.

Ultrasound waves also penetrate into the brain tissue, producing specific changes in this tissue. Academician N. N. Burdenko applied ultrasound to bring about experimental duplication of phenomena which are characteristic of a brain concussion.(9) In experiments on rabbits, he obtained a typical condition of shock. After a prolonged action of ultrasound, he observed progressive weakening of the heart activity. Death of the animal resulted from paralysis of the respiratory center.

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Injury of the central nervous system was observed when the thoracic and lumbar regions of the spinal cord of animals were exposed to the action of ultrasound waves. These experiments were carried out on mice, rats, and other animals. The injuries resulted in a loss of sensibility and in disturbances of the motor function of the hind extremities. Microscopic examination of the regions of the spinal cord that had been affected indicated disintegration of nerve cells, while the supporting tissue remained uninjured.

Injury of nerve cells and of other tissue elements was also observed when focusing lenses were used. By means of the focusing lenses it was possible to concentrate the energy furnished by the ultrasound waves at a definite depth and direct the narrow bundle of waves towards a limited region of some internal organ. This technique opens new possibilities for the utilization of ultrasound in the solution of a number of problems in the fields of biology and pathological physiology. (10)

Less thoroughly investigated were the reactions of the organism to ultrasound waves of low intensity, i.e., under conditions where a direct injury of animal tissues is not observed. This means that the so-called therapeutic intensity of ultrasound has not yet been defined on the basis of physiological and pathophysiological experiments. Nevertheless, numerous publications exist on the problem of ultrasound therapy. There are indications that therapeutic effects can be achieved by applying ultrasound waves in various diseases of internal organs of the cardiovascular system, of peripheral nerves, of joints, and of muscle tissue.

As shown above, ultrasound waves have rapidly found application in biology and medicine. Their importance in practical and theoretical microbiology cannot be doubted. Without question, the application of ultrasound techniques will find useful application in physiology and pathophysiology. The experience in investigating the biological effects of ultrasound waves will form a definite basis for further useful expansion of research in this field of science, which has not yet been adequately developed.

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